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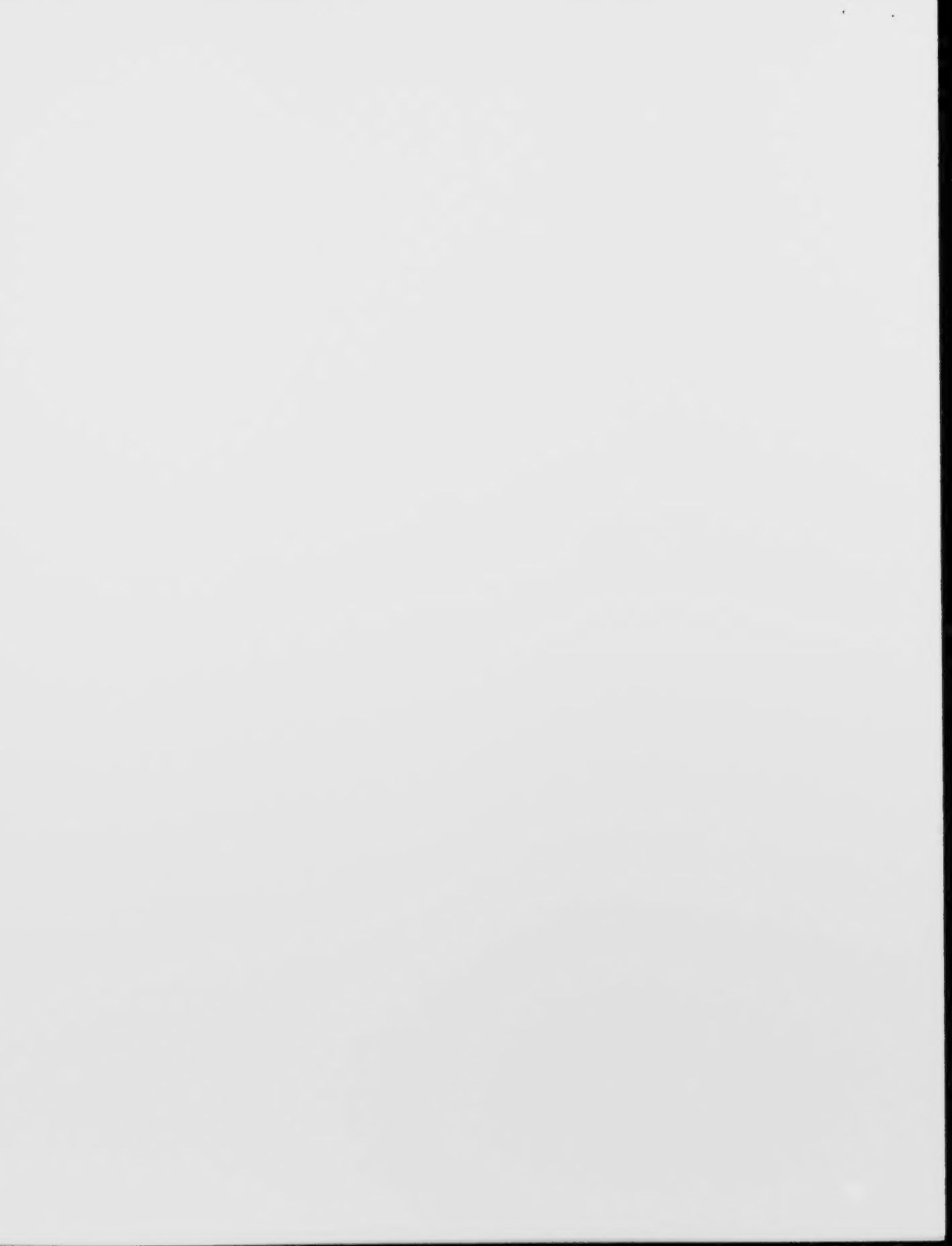
20040411

**FLAX SEED FRACTIONS AS AN ANTIBIOTIC
REPLACEMENT IN COMMERCIAL SWINE DIETS**

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Prepared by: University of Saskatchewan



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in Commercial Swine Diets**

ADF Project #20040411

Final Report

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Summary

Here we report performance results and initial microbial analysis during the first 21 days post weaning in pigs fed diets with flaxseed, flax hulls and flax oil, each at 2 inclusion rates compared with a basal diet with and without LS20 (lincomycin plus spectinomycin). The experiment was conducted using 512 pigs in two nursery rooms at PSCI Elstow. This location was specifically used to better mimic the post weaning environment and health status of typical commercial swine operations in Saskatchewan. No differences in gain, feed intake or feed efficiency, were observed among the diets over the entire 21 day period. Similarly, lactobacilli and coliform counts in feces and ileum were not affected. We did not detect salmonella shedding in feces of any pigs. Since no performance benefit was observed with antibiotic inclusion the lack of performance benefit in response to inclusion of flaxseed or flaxseed fractions is not surprising. We can conclude however, that at the levels investigated the inclusion of flaxseed fractions did not negatively performance. Indeed, the high inclusion levels of hulls and oil tested performed numerically well compared with antibiotic. In the very early postweaning period (day 0-4) there was evidence of a positive response to flax hull inclusion at 2% inclusion. A stronger challenge environment and/or weaning diets with reduced complexity and nutrient density will be required to further assess the antimicrobial potential of flaxseed or its fractions. By combining comparison of flax hulls and oil with antibiotic into a single experiment we have improved the efficiency of the study and completed most of the original project objectives in Year 1. Year 2 of the project was therefore modified to incorporate development of a Salmonella challenge and transmission model using the "Trojan Pig" approach. Construct delays limited access to level 2 biosafety animal facilities; however a single study was performed in February/March of 2007. Although no conclusion could be drawn regarding the effect of flax fractions on Salmonella

transmission, an appropriate challenge *Salmonella* isolate was selected and a model established for future investigation of diet effects.

Background and Research Motivation

The Expert Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health has recently released its report to the Canadian government recommending phasing out of antimicrobials used for growth promotion in livestock. The Canadian swine industry currently depends heavily on antibiotics for disease prevention and growth promotion. Considering that the swine industry's use of antibiotics is likely to be significantly restricted in the future, effective alternatives in management and nutrition need to be identified. Regarding flax, previous research in swine diets has primarily focused on changing fatty acid profile of pork to provide consumer health benefits; however, this use of flax seed may not directly appeal to swine producers, because pork with omega-3 fatty acids is not easily distributed separately from regular pork.

We hypothesize that the unique chemical composition of flax may give it antimicrobial properties. For example, flaxseed is a very rich in α -linolenic acid (58.5% in oil) which has been shown to inhibit the growth of bacteria in vitro, and may prevent the adhesion of bacteria to intestinal mucous and epithelial cells (Kankaanpää, 2001). In arctic charr, feeding linolenic acid promoted colonization by lactic acid bacteria, which are considered beneficial residents of the fish intestinal ecosystem (Ringo et al. 1998. *JAM*, **85**, 855). Flaxseed also contains mucilaginous water-soluble polysaccharides which can reduce feeding value due to high intestinal viscosity, although most notably in poultry. The mucilage fraction is contained mostly within the hull and consists of mainly pectic-like polysaccharides and arabinoxylan. Increased *Lactobacillus* plate counts were observed in poultry fed whole flaxseed compared to demucilaged flaxseed, suggesting that mucilage impacts microbial growth (Alzueta et al. 2003. *Brit. Poult. Sci.*, **44**, 67). Components of mucilage have been shown to stimulate the growth of beneficial *Bifidobacteria* and *Lactobacilli* in the intestine of infants and swine faecal microflora are able to effectively ferment a wide array of mucilaginous components (Smiricky-Tjardes et al. 2003. *J. Anim Sci.*, **81**, 2505). Finally, volatile fatty acids produced during the fermentation of carbohydrates in the intestine, may inhibit the growth of pathogens such as *Salmonella* (Williams et al. 2001. *Nutr. Res. Rev.*, **14**, 207)

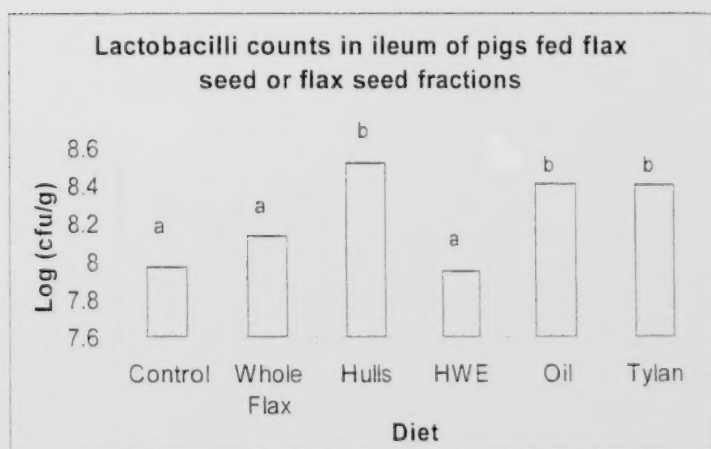


Figure 1. Mean lactobacilli counts (cfu/g) in ileal samples collected from cannulated grower pigs fed a control diet with and without tylosan or with whole flax or flax seed fractions. Bars with different letters are significantly different.

Through work initially supported by SaskPork, the Flax Council of Canada and the Crops-Livestock Interface (CLI) ADF project grant we have investigated the nutritional and antimicrobial properties of flax fractions using ileal cannulated pigs. Pigs were fed one of six diets: Diet 1: control diet based on wheat, barley and soybean meal, Diet 2: control diet with a standard antibiotic, Diet 3: control diet plus ground flax seed, Diet 4: control diet plus flax hulls, Diet 5: control diet plus hot water extracted (HWE) flax seed, Diet 6: control diet plus flaxseed oil. Using standard plate counts we observed that flax hulls and oil increased lactobacilli counts in a manner similar to the antibiotic, tylan (Figure 1). Results from the initial cannulation experiment suggested flax hulls or oil may have beneficial effects on intestinal bacterial colonization in pigs, however identification of any performance or health benefit in this study was not possible. As a result we proposed to evaluate flax hulls and oil in a commercial swine environment where sufficient numbers of pigs could be used to obtain performance data and where there is some expectation of pathogen challenge which could negatively impact performance. Results for this larger study conducted are PSCI Elstow are presented here. In addition to Saskatchewan Agriculture and Food ADF project grant #20040411, funding in partial support of this project area was also received from SaskPork in 2004/5, and Flax Council of Canada in 2005/6.

Technical Report

Flax fractions as antibiotic replacement in weaner pig diets: Production performance and pathogen shedding

Hypothesis

1. Inclusion of whole flaxseed, flax oil or hulls in the post weaning diet of pigs will improve microbial profile of the intestine lowering pathogen colonization without negative impact on performance.

Overall Objective

1. To determine the effect of flaxseed, hull and oil fractions on production performance, small intestinal microbial profile and pathogen shedding in feces of weaner pigs.

Materials and Methods

Experimental Design:

This study was conducted at a Prairie Swine Centre Elstow Inc. in order to have as near to commercial conditions as possible. The study was conducted in 2 replicates beginning on February 10 and February 24, 2005 and using 512 pigs weaned at 21 days of age. Eight Phase I and Phase II experimental diets were formulated to meet (exceed) nutrient requirements of the weaner pig (Tables 2 and 3). Diets included a control diet (CTL), control diet plus LS20 (22 mg/kg each of lincomycin and spectinomycin; ATB), control diet plus 5% ground flaxseed (FS5), control diet plus 10% ground flax seed (FS10), control diet plus 1% flax hulls (FH1), control diet plus 2% flax hulls (FH2), control diet plus 2% linseed oil (LO2) and control diet plus 4% linseed oil (LO4). The control diet (CTL) was based on wheat, soybean meal and whey powder and was formulated to contain 4% soybean oil and 2% Celite (inert). Remaining diets were formulated to near identical nutrient densities by essentially replacing soybean oil and Celite the equivalent amount of flax oil or hulls, respectively, present in the flaxseed component added. The selection of soy oil in the control diet balanced the high omega 3 content of the flax oil with a high omega 6 source allowing comparison of effect of omega3:omega6 ratio on postweaning health and performance. One tonne of each diet was mixed, pelleted, crumbled and bagged at Federated Co-op, Saskatoon. Phase I and II diets were fed postweaning days 0-7 and 8-21, respectively.

Treatment allocation:

Body weight (BW) of pigs were recorded at weaning and pigs assigned to treatment balanced for BW and stratified by body weight within treatment. For each replicate a single nursery room was used with 8 pigs allocated per pen and 4 pens per diet. Thus body weight stratification will be by quartiles. Pens will be blocked by location (4 quadrants) and assigned randomly within each quadrant to treatment.

Data and sample collection:

Pen weights were recorded and feed disappearance determined at days 0, 4, 7, 14 and 21 of the study. Fecal samples were collected aseptically from the rectum of 4 randomly selected pigs per pen on the same schedule as recording of BW. Samples from 2 pigs were combined and placed in pre-weighed sterile screwcap conical tube containing 1 mL transport medium (Peptone plus cysteine HCl) resulting in 2 pooled samples per pen. Samples were processed for enumeration of lactobacilli (MRS agar), enterobacteria-coliforms (MacConkey agar) and haemolytic aerobes (Blood agar) within 4 hours of collection. Remaining sample was enriched for Salmonella by overnight culture in selenite cysteine broth. The presence of Salmonella was confirmed by PCR analysis of the resulting pellet. On fecal collection days, pigs selected for fecal sampling were scored for fecal consistency on a 3 point scale (1=watery, 2= soft/pasty 3=normal)

Small intestinal sample collection and analysis:

One pig per pen (4 per treatment, 32 per replicate) was selected randomly and killed on day 5 postweaning by submersion in CO₂ and exsanguination. Intestinal contents were collected from the stomach, jejunum (50% of SI length), ileum (10 cm proximal to IC junction) and cecum. Viable lactobacilli and enterobacteria and haemolytic aerobe counts were determined as above for ileal contents. Ileal contents were also processed for semi-quantitative enumeration of Salmonella by enrichment and subsequent quantitative PCR. Genomic DNA will also be extracted from these samples for molecular enumeration of selected bacteria. Finally, a cross section of ileum (identified as 50 cm proximal to the IC junction) was collected in formalin for possible histological/morphological evaluation.

Statistical analysis

Performance data was analysed using a linear MIXED model with Diet, Quartile and the interaction as fixed factors and replicate as a random factor. Means for significant main effects were separated using least significant difference.

Table 2. Ingredient specifications and calculated nutrient composition of experimental Phase I diets

	CTL	ATB	FS5	FS10	FH1	FH2	LO2	LO4
Ingredient	Control - antibiotic	Control + antibiotic	Flax seed 5%	Flax seed 10%	Flax hulls 1%	Flax hulls 2%	Flax oil 2%	Flax oil 4%
Wheat	35.99	35.89	33.82	31.64	35.99	35.99	35.99	35.99
Whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean meal	15.00	15.00	15.50	16.00	15.00	15.00	15.00	15.00
Flax seed	-	-	5.00	10.00	-	-	-	-
Plasma protein	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lactose	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soy oil	4.00	4.00	2.00	-	4.00	4.00	2.00	-
Flax oil	-	-	-	-	-	-	2.00	4.00
Fish meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Blood cells	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Celite	2.00	2.00	1.00	-	1.00	-	2.00	2.00
Flax hulls	-	-	-	-	1.00	2.00	-	-
Dicalcium phosphate	1.13	1.13	0.87	0.61	1.13	1.13	1.13	1.13
Limestone	0.55	0.55	0.57	0.59	0.55	0.55	0.55	0.55
Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-threonine	0.19	0.19	0.17	0.15	0.19	0.19	0.19	0.19
DL-methionine	0.18	0.18	0.16	0.14	0.18	0.18	0.18	0.18
L-Lysine-HCl	0.14	0.14	0.10	0.05	0.14	0.14	0.14	0.14
Pellet binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
LS-20	-	0.10	-	-	-	-	-	-
Ethoxyquin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
DE	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
Crude protein	23.4	23.4		25.2	23.4	23.4	23.4	23.4
g dLys/Mcal	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
dThr/Lys	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
dTSAA/Lys	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
dMet/Lys	0.33	0.33	0.33	0.32	0.33	0.33	0.33	0.33
dTrp/Lys	0.19	0.19	0.20	0.21	0.19	0.19	0.19	0.19
Calcium	0.80	0.80	0.78	0.76	0.80	0.80	0.80	0.80
Phosphorus	0.75	0.75	0.73	0.70	0.75	0.75	0.75	0.75
Sodium	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Chloride	0.82	0.82	0.82	0.81	0.82	0.82	0.82	0.82

Table 3. Ingredient specifications and calculated nutrient composition of experimental Phase II diets

	CTL	ATB	FS5	FS10	FH1	FH2	LO2	LO4
Ingredient	Control - antibiotic	Control + antibiotic	Flax seed 5%	Flax seed 10%	Flax hulls 1%	Flax hulls 2%	Flax oil 2%	Flax oil 4%
Wheat	47.70	47.60	45.89	44.07	47.70	47.70	47.70	47.70
Soybean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Whey	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Flax seed	-	-	5.00	10.00	-	-	-	-
Soy oil	4.00	4.00	2.00	-	4.00	4.00	2.00	-
Flax oil	-	-	-	-	-	-	2.00	4.00
Celite	2.00	2.00	1.00	-	1.00	-	2.00	2.00
Flax hulls	-	-	-	-	1.00	2.00	-	-
Dicalcium phosphate	1.05	1.05	0.93	0.81	1.05	1.05	1.05	1.05
Blood cells	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fish meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.90	0.90	0.89	0.87	0.90	0.90	0.90	0.90
Vitamin premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine-HCl	0.42	0.42	0.39	0.36	0.42	0.42	0.42	0.42
L-threonine	0.30	0.30	0.28	0.26	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.18	0.18	0.19	0.20	0.18	0.18	0.18	0.18
Pellet binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
LS-20	-	0.10	-	-	-	-	-	-
Tryptophan	0.03	0.03	0.02	0.01	0.03	0.03	0.03	0.03
Ethoxyquin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
DE	3.52	3.52	3.52	3.52	3.52	3.52	3.52	3.52
Crude protein	23.0	23.0		24.5	23.0	23.0	23.0	23.0
g dLys/Mcal	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
dThr/Lys	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
dTSAA/Lys	0.56	0.56	0.58	0.59	0.56	0.56	0.56	0.56
dMet/Lys	0.34	0.34	0.35	0.36	0.34	0.34	0.34	0.34
dTrp/Lys	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Calcium	0.83	0.83	0.82	0.80	0.83	0.83	0.83	0.83
Phosphorus	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Sodium	0.31	0.31	0.32	0.32	0.31	0.31	0.31	0.31
Chloride	0.69	0.69	0.68	0.67	0.69	0.69	0.69	0.69

Results and discussion

Performance:

Average daily gain (ADG), daily feed intake (ADFI) and feed efficiency (G/F) values are given for the main effect of diet in Tables 4, 5 and 6 below. Under the conditions present at the time of this study we did not detect a performance benefit of antibiotic inclusion. Although we had selected the PSC Elstow facility as more representative of commercial pig production, the facility remains of high health status and a significant benefit of antibiotic inclusion in the early postweaning diet may not always be observed. Given the lack of effect of antibiotic on performance in this study and the overall high level of growth performance observed, the pathogen challenge was probably quite low. Thus our ability to detect any health benefit due to inclusion of flaxseed fractions based on performance was also limited. Indeed, the inclusion of whole flax seed, hulls, or oil had little impact on pig performance. Conversely, no significant negative effect of inclusion of flax in postweaning diets was observed.

Significant effects of diet on performance parameters were observed for the period from weaning to 4 days postweaning (d 0-4) and from day 4-7 postweaning, only. For the period d 0-4, the diet containing 2% flax hulls (FH2) had improved ADG and G/F. similarly the 4% flax oil (LO4) diet had numerically thigh growth rate day 0-4 and improved ($P < 0.05$) feed efficiency versus the CTL diet. For the most part, improvements in ADG and G/F observed d 0-4 were reversed d 4-7 such that no significant performance response was observed d 0-7 or for any other interval measured. However, over the entire 21 day period post weaning, the highest ADG and feed conversion efficiency was observed for the FH1, FH2 and LO4 diets.

Although no significant effect of replication on performance responses were observed, we assessed the reliability of this short term performance response immediately post weaning by comparing ADG in each replicate. Figure 4, sows the ADG from d 0-4 for each diet within replicate. In the case of the FH2 diet, the marked improvement in ADG versus the control diet was observed in both replications of the experiment giving confidence in the result. Curiously, however, and complicating explanation, the diet containing 1% flax hulls (FH1) presented the lowest ADG over the d 0-4 period in both replicates. In the case of the 4% flax oil diet (LO4), the ADG response was more variable between replications although numerically higher relative to the control diet in both.

Table 4. Effect of diet composition on average daily gain (kg/d) at intervals between 0 and 21 days after weaning

Period	Diet								Pooled SE	P
	CTL	LS20	FH1	FH2	FS5	FS10	LO2	LO4		
d 0-4	.024 ^a	.034 ^a	0.014 ^a	.110 ^b	.053 ^a	.034 ^a	.039 ^a	.082 ^a	.007	0.006
d 0-7	.090	.106	0.086	.107	.070	.101	.079	.104	.005	NS
d 0-14	.125	.146	0.115	.140	.101	.140	.120	.149	.005	NS
d 0-21	.201	.213	0.185	.210	.168	.198	.195	.230	.005	NS
d 4-7	.189 ^b	.215 ^b	.194 ^b	.105 ^a	.098 ^a	.203 ^b	.138 ^{ab}	.133 ^{ab}	.012	0.044
d 7-14	.157	.186	.146	.170	.134	.177	.167	.197	.007	NS
d 14-21	.360	.333	.334	.358	.311	.321	.350	.404	.009	NS

^{abc}Means in the same row with different superscripts are different (P < 0.05)

Table 5. Effect of diet composition on daily feed intake (kg/d) at intervals between 0 and 21 days after weaning

Period	Diet								Pooled SE	P
	CTL	LS20	FH1	FH2	FS5	FS10	LO2	LO4		
d 0-4	.089	.089	.084	.102	.080	.100	.080	.095	.003	NS
d 0-7	.121	.130	.118	.134	.111	.133	.116	.130	.003	NS
d 0-14	.178	.191	.169	.185	.163	.188	.176	.194	.004	NS
d 0-21	.266	.284	.244	.257	.246	.264	.261	.290	.006	NS
d 4-7	.167	.186	.165	.174	.157	.180	.167	.179	.004	NS
d 7-14	.241	.263	.226	.244	.224	.247	.245	.275	.007	NS
d 14-21	.454	.471	.401	.424	.419	.423	.443	.482	.010	NS

^{abc}Means in the same row with different superscripts are different (P < 0.05)

Table 6. Effect of diet composition on feed efficiency (G/F) at intervals between 0 and 21 days after weaning

Period	Diet								Pooled SE	P
	CTL	LS20	FH1	FH2	FS5	FS10	LO2	LO4		
d 0-4	.170 ^a	.266 ^a	.159 ^a	1.091 ^b	.502 ^{ab}	.280 ^{ab}	.401 ^{ab}	.789 ^b	.094	0.039
d 0-7	.738	.824	.729	.812	.556	.771	.676	.798	.034	NS
d 0-14	.684	.777	.685	.752	.546	.739	.681	.771	.021	NS
d 0-21	.763	.748	.766	.803	.676	.748	.741	.800	.011	NS
d 4-7	1.111 ^b	1.141 ^b	1.138 ^b	.593 ^a	.563 ^a	1.123 ^a	.850 ^{ab}	.760 ^{ab}	.062	0.018
d 7-14	.648	.753	.668	.720	.541	.720	.685	.721	.026	NS
d 14-21	.794	.715	.846	.834	.756	.751	.790	.846	.018	NS

^{abc}Means in the same row with different superscripts are different (P < 0.05)

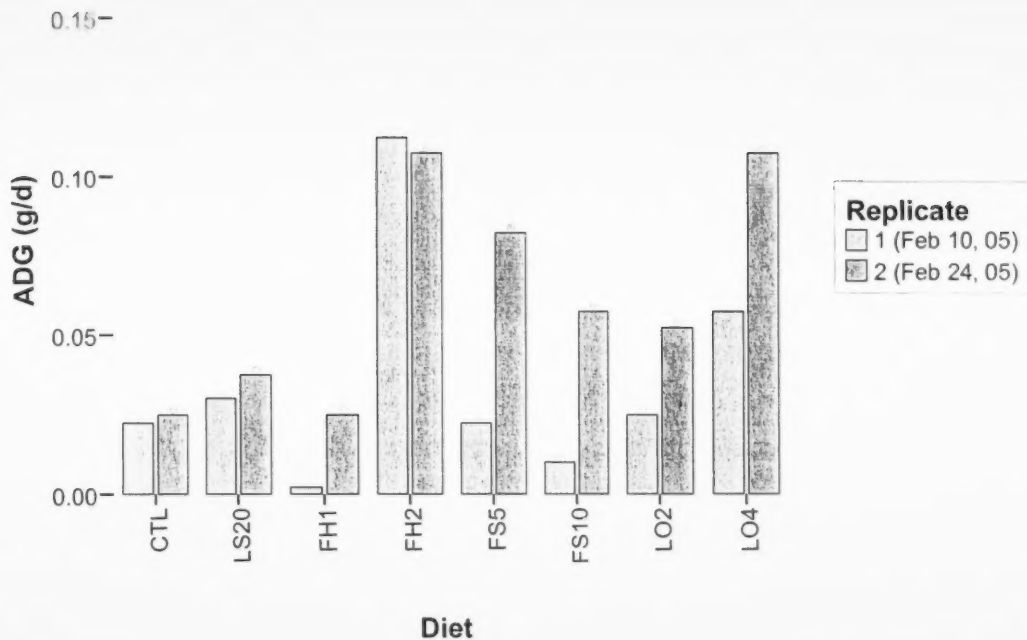


Figure 4. Effect of diet composition on average daily gain (kg/d) between 0 and 4 days after weaning. Bars represent mean ($\pm$ SE) gain for each replication.

Fecal microbial counts

Total fecal lactobacilli and coliform data obtained using standard plate counts methods has been summarized. Figures 5 and 6 show the number (log CFU/g) of lactobacilli and coliforms enumerated in feces (samples from 2 pigs pooled; 4 pigs sampled per pen) at regular intervals over the 21 day postweaning period. Lactobacilli and coliform counts varied by less the 0.5 log CFU/g feces among diets. Although a statistical comparison has not been completed the variation is unremarkable and unlikely to be biologically significant. There was a marked decline in coliforms on day 21 in all pigs associated with a smaller elevation in lactobacilli. The change probably reflects a postweaning transition and stabilization of microbial colonization which has been reported previously. The decline in coliforms at day 21 was very dramatic in the first replicate (approx. 3.2 logs) but also evident in replicate 2 (approx. 1.0 log).

Molecular analyses of pathogen colonization in ileum and shedding in feces is on going. TO date we have not been able to detect salmonellas by PCR in either enriched fecal or ileal digesta samples.

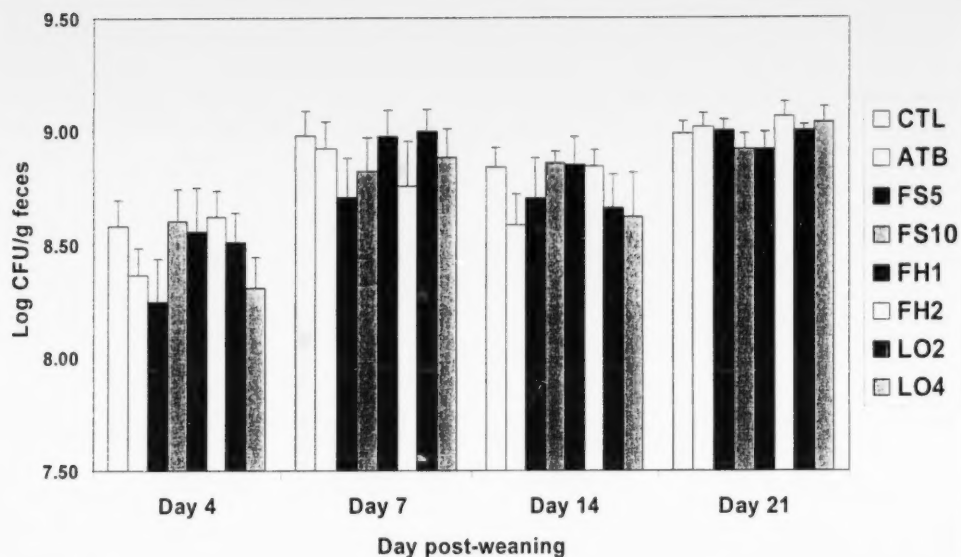


Figure 5. Effect of diet composition on mean ($\pm$ SE) lactobacilli counts (CFU/g) in fecal samples collected at regular intervals.

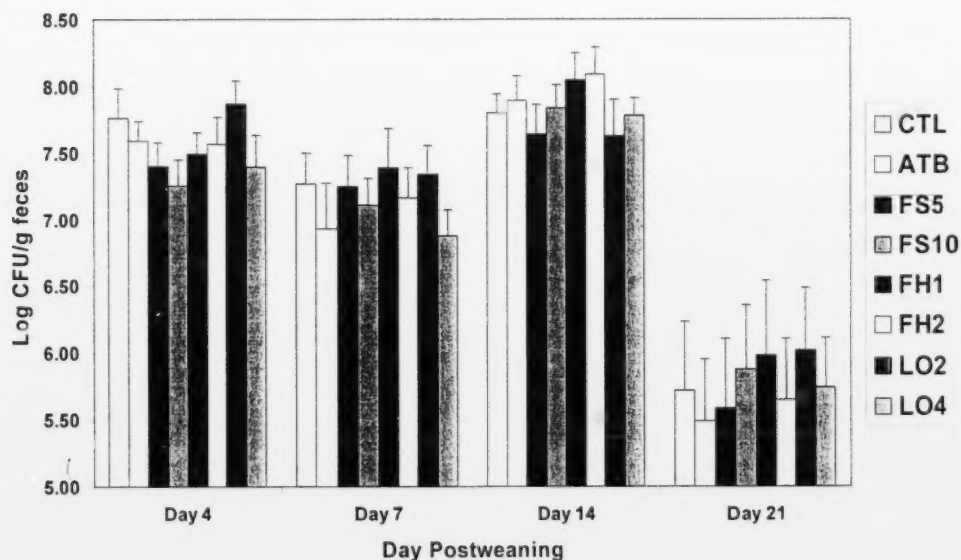


Figure 6. Effect of diet composition on mean ($\pm$ SE) coliform counts (CFU/g) in fecal samples collected at regular intervals.

Ileal microbial counts

Ileal contents was collected from 1 pig per pen in each replicate experiment 5 days after weaning. Again only the lactobacilli and coliform data obtained by standard plate counting have been summarized (Figure 7). No significant effect of diet was observed. These results contrast our previous findings in the cannulated growing pig model in which the inclusion of 4% flax hulls or 8% oil increased lactobacilli counts in ileum in a manner similar to that observed when tylosan was added to the basal diet. Differences between the studies that could account for the different response include the use of weaner pigs fed experimental diets for only 5 days postweaning in the current study versus growing pigs fed experimental diets for 15 days in the previous experiment. Also the inclusion rates of hulls and oil were higher in the grower pig study.

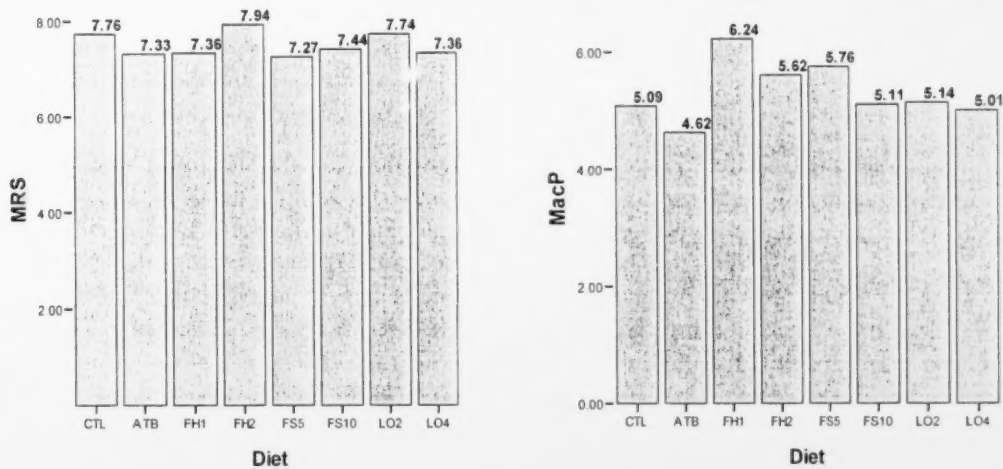


Figure 7. Effect of diet composition on mean ($\pm$ SE) lactobacilli (MRS) and coliform (MacP) CFU/g ileal contents collected 5 days post weaning from 1 pig per pen in each replicate experiment.

Development of a Salmonella challenge model determination of effect of flax fractions on Salmonella transmission in weanling pigs.

Hypothesis

1. Inclusion of flax oil or hulls in the post weaning diet of pigs will reduce transmission of salmonella among pigs.

Overall Objectives

1. To develop a salmonella challenge model to study effect of diet composition and additives on transmission of salmonella among pigs
2. To determine the effect of hull and oil fractions on transmission of salmonella among weaner pigs.

Background

This experiment was added to the research plan for this project with permission of the Mr. Bob Drysdale, SAF Program Manager for livestock. Justification for adding this component to the research plan was based on the ability to conduct a single large experiment as reported above examining 2 inclusion rates of flaxseed, hulls and oil. This had significant cost saving versus the original proposal in which separate experiments for Flax hulls and oil were proposed. Conduct of a Salmonella challenge experiment requires animal facilities with level 2 biosafety approval. These facilities were under construction at WCVN at the time the proposal amendment was contemplated with a completion date scheduled for spring 2006. Unfortunately, as a result of construction delays the facility did not receive level 2 biosafety approval until mid February of 2007. The experiment reported here began Feb 16, 2007 and was completed on March 8, 2007. Construction delays necessitated the 6 month extension to the project (approved by Dr. H. Soita, current SAF Program Manager for livestock) and limited our ability to conduct significant model testing.

Materials and Methods

Experimental Design:

105 weanling pigs (21 days of age) were purchased and allocated to 15 pens at 7 pigs per pen located in the level 2 biosecurity animal facilities (BSL Level II unit) at WCVN. Each pen contained a nipple drinker, rubber floor mats and a gravity feeder. The lighting program was 12hL:12hD and room temperature was maintained 32 °C at the beginning of the study and reduced by 1 °C every other per day until 28 °C.

Pigs in each pen were assigned to one of 5 experimental diets formulated as provided in Table 3 (Phase II diets) above. Specific diets included in the challenge trial were CTL (no antibiotic), flax hulls at 1 and 2 % inclusion (FH1, FH2), and flax oil at 2 and 4 % inclusion (LO2, LO4). Diets were fed ad libitum from the day of weaning until study completion.

Challenge Organisms:

Salmonella isolates used to generate challenge organisms for the present study were obtained from Dr. Cheryl Waldner (WCVM) and Dr. Margaret McFall (Laboratory Scientist, Agri-Food Laboratories Branch, Alberta Agriculture). The isolates were generated as part of a western Canadian survey of salmonella prevalence in swine herds. Five antibiotic sensitive serotypes obtained from swine feces were provided including *Salmonella enterica* subsp. *enterica* serotypes Derby, Infantis, Mbandaka, Putten and Typhimurium var Copenhagen. Subsequently, we cultured all available isolates and selected for natural mutants resistant to both novobiocin and naladixic acid (each at 25 ug/mL). By selection for resistance for these antibiotics we could more easily recover the isolate in feces of challenged pigs.

Challenge Protocol:

For *Salmonella* challenge, at 5 days after weaning, two pigs were randomly selected from each pen were placed in a separate pen and designated "Trojan pigs". The remaining 5 pigs per pen were designated "Contact pigs". Oreo cookies were soaked in a fresh overnight broth culture of $\text{Nal}^r/\text{Nov}^r$ *Salmonella enterica* subsp. *enterica* serotypes Derby such that each cookie contained approximately 10^{10} cfu salmonella. Trojan pigs were offered the Oreo cookie and were kept isolated from their contact penmates for a period of 5 hours without access to feed. Trojan pigs were then return to the original pens.

A second salmonella challenge was performed 12 days after weaning and 7 days after the initial challenge. This second challenge was conducted to test colonization and transmission $\text{Nal}^r/\text{Nov}^r$ *Salmonella enterica* subsp. *enterica* serotypes Putten and Typhimurium var Copenhagen. Since each diet was replicated in 3 pens this second challenge 1 group of 5 pens representing all 5 diets was not re-challenged, one group of 5 pens was challenged with $\text{Nal}^r/\text{Nov}^r$ *Salmonella enterica* subsp. *enterica* serotypes Putten and one group of 5 pens was challenge with $\text{Nal}^r/\text{Nov}^r$ *Salmonella enterica* subsp. *enterica* serotypes Typhimurium var Copenhagen. POigs selected as Trojan pigs in the first challenge were not selected as Trojan pigs in the second challenge. The challenge protocol for Trojan pigs was the same, except that the organism was delivered by syringe to the back of the throat of each Trojan pig (10^{10} cfu in 5 mL culture).

Fecal Sampling and Salmonella enumeration:

Fecal samples were collected at regular intervals following challenge from both trijan anf Contact pigs. Fecal samples were either diluted and plated directly on Brilliant Green Agar containing novobiocin and naladixic acid each at 25 ug/mL (sensitivity 10^2 cfu/g feces) or enriched overnight in selenite cysteine broth containing novobiocin and naladixic acid (each at 25 ug/mL). Results are reported as +++, ++, + or negative.

Results and Discussion

We chose to develop a "Trojan" pig challenge model in order to more closely mimic the natural transmission of salmonella between pigs and thus provide a better assessment of dietary treatments on transmission. The first objective was to develop the challenge model and the second to assess the effect of flax fractions on salmonella transmission.

We would have preferred to conduct smaller scale studies to develop the challenge model prior to conducting a full test of diets containing flax. Unfortunately, due to the delay in availability of BSL Level II animal facilities we elected to proceed with the full study. Regarding

development of the model, Nal^r/Nov^r *Salmonella enterica* subsp. *enterica* serotype Derby was selected for the initial challenge. The day after challenge only 2 of 6 Trojan pigs fed the control, FH2, LO2 and LO4 diets and 3 of 6 Trojan pigs fed the FH1 had detectable *Salmonella* in feces by direct plating. Two days after challenge Nal^r/Nov^r *Salmonella* was detectable by direct plating in only 1 Trojan pig in the FH4 treatment group. NO Trojan pigs were positive by direct plating on day 5 following challenge. On day 7 following challenge, fecal samples were enriched overnight followed by plating in order to determine whether pigs were shedding salmonella at low levels. Among Trojan pigs, 1 of 6 pigs fed CTL, FH1, FH2 and LO4 diets and 2 of 6 Trojan pigs fed the LO2 diet were positive for Nal^r/Nov^r *Salmonella*. Among Contact pigs, 2 of 15 fed CTL, FH1 were positive and 3 of 15 contact pigs fed the LO2 diet were positive. These results suggested the challenge serotype (Derby) was a surprisingly low colonizer in the pig and unsuitable for the challenge study. No conclusions regarding the effect of diet composition could be drawn.

In order to try to obtain useful information from this study we elected to perform a second challenge using different challenge serotypes to test their colonization and transmission characteristics. Two additional serotypes were selected (Putten and Typhimurium var Copenhagen) and challenge occurred on day 12 after weaning (day 7 after first challenge). Since each diet was replicated in 3 pens, we left one pen challenged (challenged with Derby only during 1st challenge), and each of the remaining 2 pens challenged with one of the new serotypes. Different Trojan pigs were selected. Direct plate counts for plate counts Nal^r/Nov^r *Salmonella* in feces were conducted 2 days after the second challenge. An overnight enrichment was conducted on day 5 after challenge. None of the pigs challenged with Derby only were positive for Nal^r/Nov^r *Salmonella* after direct plating on day 2. On day 5, after enrichment only 4 pigs were positive for Nal^r/Nov^r *Salmonella*; 1 in FH1, 1 in FH2 and 2 in LO2 treatment groups. For pigs challenged with the Putten serotype, only Trojan pigs were positive by direct plate count on day 2 post challenge. Both Trojan pigs in the FH1 group were positive and 1 of 2 Trojan pigs in each of the FH2, LO2 and LO4 diet groups. On day 5 after enrichment, no Trojan pigs were positive but 3 contact pigs were positive; 1 CTL contact, 1 FH1 contact and 2 LO2 contact pigs. For pigs challenge with the Typhimurium serotype direct plate counts on day 2 showed all Trojan pigs were positive with exception of 1 CTL Trojan and 1 FH1 Trojan. Some contact pigs were also positive by direct count including 2 CTL contacts, 1 FH1 contact, and 1 LO4 contact. On day 7, after enrichment all pigs were positive for Nal^r/Nov^r *Salmonella* with exception of 3 CON contact pigs, and 2 LO2 Contact pigs. We conclude that the Nal^r/Nov^r *Salmonella* Typhimurium var Copenhagen is a suitable isolate for future use in the model. We also conclude that both direct plating and enrichment will provide a useful assessment transmission of salmonella following challenge. We can unfortunately draw conclusions regarding the effect of flax fractions on salmonella transmission.

Personnel

Graduate students Laura Smith and Angie Goldfinch have used samples collected in these experiments towards completion of M.Sc. degrees. Mr. Jason Marshall was the primary technical staff responsible for sourcing diet ingredients, diet preparation and study organization/conduct and microbial enumeration.

Project Developed Materials

Nal^r/Nov^r *Salmonella enterica* subsp. *enterica* serotypes Derby, Infantis, Mbandaka, Putten and Typhimurium var Copenhagen.

Project Photos**Acknowledgement**

Part of this work was presented at the annual Meeting of the American Society of Animal Science in St. Louis MO in July 2004, and at an invited presentation at the Mid-Western Section Annual Meeting of the American Society of Animal Science in March 2005. Results presented in this report have been presented and student poster presentations in the College of Agriculture (Animal and Poultry Science Graduate Student Poster Presentations) and University of Saskatchewan (Life Science conference). We anticipate further publication of results in the PSCI annual report and as peer reviewed publications. Acknowledgement to all sponsors including SaskPork, Saskatchewan Agriculture and Food ADF and Flax Council of Canada was and will be noted.

